

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Applicant:	Watkins, Jeffry D.	Group Art Unit:	1644
Serial No.:	10/553,938	Examiner:	Ron Schwadron, Ph.D.
Application Date:	October 21, 2005	Confirmation No.:	8652
For:	CD20 Binding Molecules		
Docket No.:	X-16760A		

DECLARATION OF APPLICANT UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jeffry D. Watkins, hereby declare the following:

A duly executed Declaration and Power of Attorney was filed on October 21, 2005 in the above-referenced patent application, declaring that Julian Davies, David M. Marquis, Barrett W. Allan, Brian Ondek, and myself are the original and first inventors of the subject matter which is claimed and for which a patent is sought in the above-referenced patent application, and the same is true and correct.

I understand that a Business Wire article entitled "Applied Molecular Evolution Advances Optimized Versions of anti-TNF alpha and anti-CD20 Monoclonal Antibody Therapeutic Candidates", dated January 3, 2003 (hereinafter, the "Business Wire reference") has been cited by the Examiner in the above-referenced patent application as anticipating the pending claims of the same.

I have also read and understand the Business Wire reference. In relevant part, the Business Wire reference describes, in entirely functional terms, a CD20 binding antibody,

AME-133, reported to have improved functional attributes as compared to Rituxan®, a therapeutic CD20 binding antibody known and commercialized at the time of the publication of the Business Wire reference.

I also understand that the above-referenced patent application presently claims compositions comprising a CD20 binding molecule (e.g., antibodies or CD20 binding fragment thereof) comprising a set of three structurally defined heavy chain CDRs and a set of three structurally defined light chain CDRs.

The invention presently claimed by the above-referenced patent application was based on detailed experiments involving antibody optimization which resulted in functionally improved CD20 binding antibodies comprising the CDRs defined by specific amino acid sequences.

This declaration is to establish the actual reduction to practice in the United States of the invention claimed in the above-referenced patent application, at a day prior to January 3, 2003, which is the effective date of the Business Wire reference.

At the relevant time of the actual reduction to practice of the invention claimed by the above-referenced application, I held the position of Chief Scientific Officer at Applied Molecular Evolution (AME), Inc.

The invention disclosed and claimed in the instant patent application was reduced to practice (in the United States) prior to January 3, 2003, as is evidenced by the attached Exhibits, which are more fully described below.

To the best of my knowledge and belief, the claimed invention was not sold or in public use in the United States for one year prior to the date of the above application.

To the best of my knowledge and belief, the claimed invention was not patented nor described in a printed publication in such a manner that a person of ordinary skill in the field of the invention would have been able to make or use the claimed invention, without undue experimentation, prior to the date of the above application.

The attached Exhibits were generated and/or prepared by AME employees, including Christine Hawelka and Ying Nie, who are not inventors of the claimed subject matter, while recording and/or documenting work conducted (in the United States) under the direction and supervision of myself and/or the other inventors listed on the Declaration and Power of Attorney filed on October 21, 2005.

The following documents are submitted as evidence establishing the date of completion of the claimed invention of the above-referenced application as being prior to January 3, 2003.

- a. Copies of sequence files generated with Sequencher™ (Gene Codes Corp.) on October 10, 2002 showing sequences encoding heavy chain variable regions and light chain variable regions of CD20 binding molecules, including those of AME 33 (Exhibits 1-4).
- b. Copies of various pages from Christine Hawelka's AME Research Notebook #585, dated from October 9, 2002 to October 17, 2002 (Exhibit 5).
- c. Copies of the cover page and page 19 (dated November 4, 2002) from Ying Nie's AME Research Notebook #613 (Exhibit 6).

The documents submitted as Exhibits 1-6 clearly demonstrate that the presently claimed invention was reduced to practice (in the United States) on or before October 10, 2002.

The documents submitted as Exhibits 1-6 clearly demonstrates that the presently claimed invention was reduced to practice (in the United States) prior to January 3, 2003, the effective date of the Business Wire reference.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willfully false statements may jeopardize the validity of this application or any patent issuing thereon.


Jeffrey D. Watkins

June 29, 2011
Date

DESCRIPTION OF THE EXHIBITS

Exhibit 1

A copy of an electronic sequence file generated with SequencherTM software (Gene Codes Corp.) on October 10, 2002 showing the sequences (both nucleotide and amino acid sequences) obtained from DNA sequencing of the DNA encoding the heavy chain variable regions of various CD20 binding molecules. In particular, the nucleotide sequence encoding the heavy chain variable region of the CD20 binding molecule AME 33 is shown as the fourth nucleotide sequence down from the top of the page (i.e., 1_DM.33.530_F04_12.abl). Directly below the fourth DNA sequence is the amino acid sequence (provided in conventional single-letter code) of the heavy chain variable region of the CD20 binding molecule AME 33.

Exhibit 2

A copy of an electronic sequence file generated with SequencherTM software (Gene Codes Corp.) on October 10, 2002 showing the sequences (both nucleotide and amino acid sequences) obtained from DNA sequencing of the DNA encoding the light chain variable regions of various CD20 binding molecules. In particular, the nucleotide sequence encoding the light chain variable region of the CD20 binding molecule AME 33 is shown as the fourth nucleotide sequence down from the top of the page (i.e., 1_DM.33.355_F05_11.abl). Directly below the fourth DNA sequence is the amino acid sequence (provided in conventional single-letter code) of the light chain variable region of the CD20 binding molecule AME 33.

Exhibit 3

A marked up version of Exhibit 1 has been provided here as Exhibit 3 for the Examiner's convenience.

The amino acid sequences of CDRH1, 2, and 3 of the CD20 binding molecule AME 33 are underlined at page 2, 3, 4 and 5, respectively, of Exhibit 3. The underlined amino acid sequences of CDRH1, 2, and 3 correspond exactly to SEQ ID NOs: 25, 39, and 57, respectively, of the above-referenced application. Importantly, SEQ ID NOs: 25, 39, and 57 are all recited elements in present claim 34.

Furthermore, in Exhibit 3, the amino acid sequence of the entire heavy chain variable region of the CD20 binding molecule AME 33 has been enclosed by brackets (starting at page 1 and ending at page 5). The entire heavy chain variable region of the CD20 binding molecule AME 33 (shown enclosed in brackets) corresponds exactly to SEQ ID NO: 61 of the above-referenced application. Importantly, SEQ ID NO:61 is a recited element in present claim 48.

Exhibit 4

A marked-up version of Exhibit 2 has been provided here as Exhibit 4 for the Examiner's convenience.

The amino acid sequences of CDRL1, 2, and 3 of the CD20 binding molecule AME 33 are underlined at page 2, 3, and 4, respectively, of Exhibit 4. The underlined amino acid sequences of CDRL1, 2, and 3 correspond exactly to SEQ ID NOs: 5, 13, and 19, respectively, of the above-referenced application. Importantly, SEQ ID NOs: 5, 13, and 19 are all recited elements in present claim 34.

Furthermore, in Exhibit 4, the amino acid sequence of the entire light chain variable region of the CD20 binding molecule AME 33 has been enclosed by brackets (starting at page 1 and ending at page 5). The entire light chain variable region of the CD20 binding molecule AME 33 (shown enclosed in brackets) corresponds exactly to SEQ ID NO:59 of the above-referenced application. Importantly, SEQ ID NO:59 is a recited element in present claim 48.

Exhibit 5

A copy of the cover page, table of contents page, and various other pages from Christine Hawelka's AME Research Notebook #585, dated from October 9, 2002 to October 17, 2002, are provided as Exhibit 5. Throughout these research notebook pages, references to "33", or variations thereof (such as "#33", "33 F1", etc.), are references to the CD20 binding molecule referred to as AME 33 in the above-referenced application.

Page 66 of Christine Hawelka's AME Research Notebook #585, indicates, *inter alia*, that David Marquis ("Dave") set up and finished high-titer ("HT") and ("single-strand") preps of various clones ("31-40"), including clone 33, on October 8, 2002 and October 9, 2002, respectively.

Page 68 of Christine Hawelka's AME Research Notebook #585, describes, *inter alia*, a fixed Ramos cell ELISA binding assay with various CD20 binding molecules, including Fab 33. It is noted on this notebook page, dated October 10, 2002, that "#32, 33, 35, [and] 40 look the best, so will investigate them further". For the avoidance of doubt, the references to "33" or "reference is referred to as AME 33, Fab AME 33 or the like in the present application.

Page 73 of Christine Hawelka's AME Research Notebook #585, indicates, *inter alia*, a fixed Ramos cell ELISA binding assay with various CD20 binding molecules, including the Fab AME 33. The page, dated October 17, 2002, notes that after 18 hours, "Brian's 4H5 as well as 33 [and] 40 staying on pretty well".

Exhibit 6

A copy of the cover page and page 19 Ying Nie's AME Research Notebook #613 are provided as Exhibit 6. Page 19 of Ying Nie's AME Research Notebook #613, dated November 4, 2002, documents the purification of single-strand DNA ("ssDNA") encoding the light chain variable region of clone 33 ("VL33") and the heavy chain variable region of clone 33 ("VH33"). "VL33" and "VH33" on page 19 of this notebook are refer to DNA molecules encoding the light chain variable region and heavy chain variable region, respectively, of the CD20 binding molecule referred to as AME 33 in the above-referenced application.

ContigVH
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EXHIBIT 1

PAGE 4


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[illegible]

ContigVL
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ContigVH
Sequencher (cm) '+'s 1d10 combi 10-10-02.SPF*

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EXHIBIT 3

PAGE 3

ConfigVH
Sequencer (nm) *+s 1D10 combi 10-10-02.SPR"

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ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_32_530_G04_14.ab1
ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_33_530_F04_12.ab1
ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_34_530_E04_10.ab1
ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_35_530_D04_08.ab1
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I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_38_530_C04_06.ab1
ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_39_530_B04_04.ab1
ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

#271 1_DM_40_530_A04_02.ab1
ATCTCAGCCGCAAGTGCATTCAGCACCGCCTACCTGCGAGTGGAGAGACCTTGAAGCCCTGCGACACCCGCCATGTATTTACTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

```

EXHIBIT 3

PAGE 4

ConfigVH
Sequencher (cm) *+s 1d10 combi 10-10-02.SPF"

#1010 VH	#361	ACTTACGTGGCGGTGACTGGACTTTCGATGTCGTGGGCAAAAGGACCACGGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W T F D V W G K G T T V T V S S A S T K G
#1.DK.11.530_H04_16.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#1.DK.12.530_G04_14.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#1.DK.13.530_F04_12.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#1.DK.14.530_E04_10.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W T F D V W G K G T T V T V S S A S T K G
#1.DK.15.530_D04_08.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#1.DK.18.530_C04_06.ab1	#361	TAATACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	Y	X V G G D W Q F D V W G K G T T V T V S S A S T K G
#1.DK.19.530_B04_04.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W T F D V W G K G T T V T V S S A S T K G
#1.DK.40.530_A04_02.ab1	#361	ACTTACGTGGCGGTGACTGGAAGTTCGATGTCGTGGGCAAAAGGACCACCGTCAACCGTCCCTCAAGCCCTCCACCAAGAGGCC
	T	Y V G G D W Q F D V W G K G T T V T V S S A S T K G

EXHIBIT 3

PAGE 5

ContigVL
Sequencher (cm) *+s ID10 combi 10-10-02.SPF*

#1 ID10 VL
#2 1_DM, 31, 355, PD05, 15, ab1
#3 1_DM, 32, 355, PD05, 13, ab1
#4 1_DM, 33, 355, PD05, 11, ab1
#5 1_DM, 34, 355, PD05, 09, ab1
#6 1_DM, 35, 355, PD05, 07, ab1
#7 1_DM, 38, 355, PD05, 05, ab1
#8 1_DM, 39, 355, PD05, 03, ab1
#9 1_DM, 40, 355, PD05, 01, ab1

```

#1 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P L L P T P V A K A E I V L T Q S P G T
#2 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#3 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#4 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#5 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#6 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#7 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#8 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T
#9 ATGAAGCAGTCTACCATTTGCACTTACCGCTTTATTCATCCCGTTCGCAAGCGGAATTTGATGACCGCAGTCTCCAGGCACC
M K Q S T I A L L P L L P T P V A K A E I V L T Q S P G T

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ContigVL
Sequencer (cm) *+s ID10 combi 10-10-02.SPF*

#175 ID10 VL
G G C C A G G C T C C C A G G C T C T C A T C T A T A T G C C A A T C C A C C T G G C T T C G C A T C C C A G A C A G G T T C A T A T G G A T G G C T G G C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
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G G C C A G G C T C C C A G G C T C T C A T C T A T A T G C C A C C T G C A C C T C T G C A T C C C A G A C A G G T T C A T A T G C A G T G G C T G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
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G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
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G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
#175 1_DM_34_355_E05_09.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T A T G C C A C C C A C C C T G C A T C C C A G A C A C A G G T T C A T A T G C A T G G C T G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
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G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
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G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
#175 1_DM_40_355_A05_01.ab1
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G Q A P R L L I Y A T S N L A S G I P D R F S G S G T

ContigVL
Sequencher (tm) "+"s 1d10 combi 10-10-02.SPF"

```

#262 1D10 VL
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D F T L T I S R L E P E D F A V Y C Q Q W L S N P P T F
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D F T L T I S R L E P E D F A V Y C Q Q W L S N P P T F
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#262 1.DM.34.355_E05_09.ab1
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EXHIBIT 5

P29-97 CD20 8-22-02 to 11-7-02

NOTEBOOK NO. 585
ISSUED TO Chris Hawelka
ON 6-11 2002
DEPARTMENT _____
RETURNED _____ 20 _____

SCIENTIFIC NOTEBOOK COMPANY
2831 LAWRENCE AVENUE
STEVENSVILLE, MICHIGAN 49127
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Table of Contents

Page

This Notebook contains info on projects

CO-20 P. 28-97

From Page No. _____

10/8 Antigen peni prep on 31-40

elute 15ml TE

dilute peni prep 1:3 7/12 BSA-PBS

+ pen assay CI #31-40

10/8 Done set up HT+SS on 31-40

31 E2 36 F4

32 E2 37 DT

33 F1 38 B1

34 G1 39 F4

35 B1 40 C9

Finished on 10/9

10/9 Error XLO's for 15ml peni prep

300 ml 2xYT 9-28

300 ml Tet

3 ml XLO 10/9

mix + grow @ 37°C shaker 0.903

Check OD₅₉₅ 2.283 pm 0.898

Add 300 ml 1M IPTG + peni mix

pilot 15ml fraction

Add 5 ml HT from #31-40 above

+ New Chi

LI-1010

Biomix 4H5

10/9 Set 3 Remo. plates

Dead 3pm = $13 \frac{1}{2} \times 2 \times 2 (1204) = 1.3 \times 10^5$ deadlive $506 + 649 = 1155 - 13 = (122/6 \times 10^4) 1204 = 1.1 \times 10^7$

$$\frac{1.3 \times 10^5}{1.1 \times 10^7} = \frac{1.19}{11} \left[(1.0118/19) \times 100 \right] - 100 \quad \{99.2\% \text{ viability}\}$$

Set 3 Quant plates

10ml Carbonate Buffer + 3.92 x fd mix + pipet 50 µl each

to poly D type plate shake @ 4°C ON

To Page No. _____

Witnessed & Understood by me.

Date

Initialed by

Christine M. Handley

Date

10-9-82

Recorded by

Quant	
Chi	12.90
21010	13.02
LI-1010	27.12
Biomix 4H5	7.58
17	55.94
15	38.35
17	32.96
18	42.59
17	48.50
21	66.54
22	52.25
23	43.68
24	37.06
25	41.71
26	69.83
27	45.57
28	42.52
29	59.73
Chi	12.95
LI-1010	38.84

From Page No.

7/4/10 Finished 15ml series for #30-40

Did 2 Rammed Aggregate + 3 Granite

Granite based 100 fold dilution program - target absolute Fab Core #2. } NOTE

New Chl - 25.00 mg/ml / 25.00

61-1D10 59.02 29.51

30-H1 19.85 7.425

31-E2 89.05 44.525

32-E2 59.44 29.72

33-F1 44.51 22.255

34-G1 74.48 37.24

35-B1 71.75 35.875

36-F1 70.86 35.43

110-20 110.20 55.10

37-D8 75.12 mg/ml

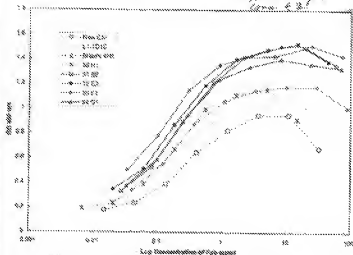
38-B1 88.77 44.385

39-F4 68.42 34.21

New Chl 40.08 20.04

40-C9 105.28 52.64

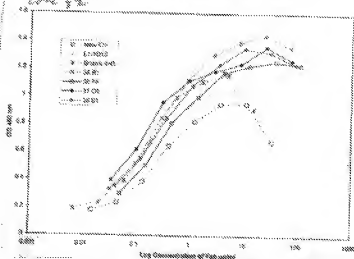
Binding of Fab to Fixed Rammed Cells 10-10-02

granite fab
core #2

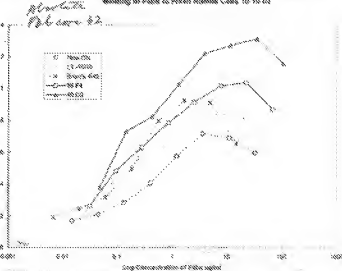
1010 is slightly above. Chl in all these graphs didn't show binding (its in yellow) - converted - plotted and new graphs.

#32, 33, 35 + 40 looks the best so will investigate them further

Binding of Fab to Fixed Rammed Cells 10-10-02

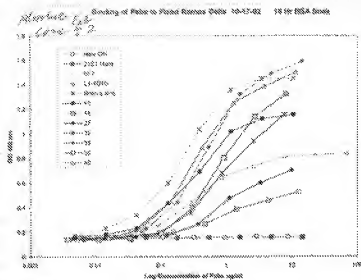


Binding of Fab to Fixed Rammed Cells 10-15-02



To Page No.

From Page No. _____



- 18 hr - Shows *Kanine 9461* as well as *27* staying on pretty well

- *Chimerin*, *6FI* + *21E1* have off

- *18* has come off the most

- *27* + *1010* have also fallen off a little

10/17 *Kanine 9461* *D101N* *65 mM* *841 ng/ml*

template ~ 9000 bases $\times 330 = 2,970,000$ M

oligo 39 bases $\times 330 = 12,870$ M

$2,970,000 / 12,870 = 230.77$ or 231×5

$9000 / 39 = 230$'s too much if 160 ng each

9000 template	160 ng	160 ng	160 ng	160 ng	160 ng
39 oligo	160 ng	160 ng	8 ng	7 ng	7 ng
too much	230's too much	230's	11.5X	10X	about right

So want 5 ng of oligo in 12 \times 20 \times 20 \times 5 ng = 100 ng. ~ 100 ng

conc. $841 \text{ ng/ml} \Rightarrow 841 \text{ ng}/\mu\text{l}$ $1:60$ dilution of stock H₂O 140 ng ~ 100 ng

2.2 10X Phosphatase Buffer 20 \times total
 1.2 10X ATP 4-15 \times
 1.66 \times oligo *9461*
 14.8 \times *50* \times *14.6*
 5.2 *Kanine*
 1 hr @ 37°C

To Page No. _____

Witnessed & Understood by me.

Date

Prepared by

Christine M. Hirsch

Recorded by

Date

10-17-02

NOTEBOOK NO. 613
ISSUED TO Ying Nie
ON 9/17/02 20
DEPARTMENT _____
RETURNED _____ 20

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2831 LAWRENCE AVENUE
STEVENSVILLE, MICHIGAN 49127
(800) 537-3028 - <http://www.snco.com>

From Page No.

1. get PCR production from YT
~100 μ l/sample

VH \leq 18
33

VL \leq 18
33

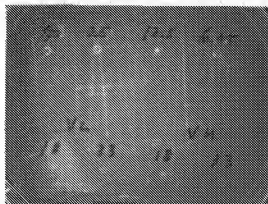


2. PCR product purification
- +500 μ l buf 1 (binding buf)
to PCR product \rightarrow column
 - +500 μ l buf 2 (washing buf) \times 2
+200 μ l
 - +50 μ l buf 3 (elute buf) \times 2
then +150 μ l dH₂O

3. to each sample.

- 150 μ l of Magnetic beads + 400 μ l 2x R&W buf — wash \times 2
resuspend in 200 μ l 2x R&W buf
- beads + DNA gently shaking 16'
- wash beads w/ 2x R&W buf \times 4
- elute DNA in 30 μ l of 0.15M NaOH, gently shaking 10'
- take out, \times to remove residual beads
- + 1 μ l glycogen
- 30 μ l 3M NaOAc (pH 5.0) / -80°C — 30'
- 800 μ l 100% EtOH
- \times , 70% EtOH wash, vac dry, dissolve in 15 μ l dH₂O

keep at -20°C (YT refr.)



To Page No.

Witnessed & Understood by me

Date

Invented by

Date

Carolyn Ho

6.1.03

Recorded by

Ying Nie

11/4/02